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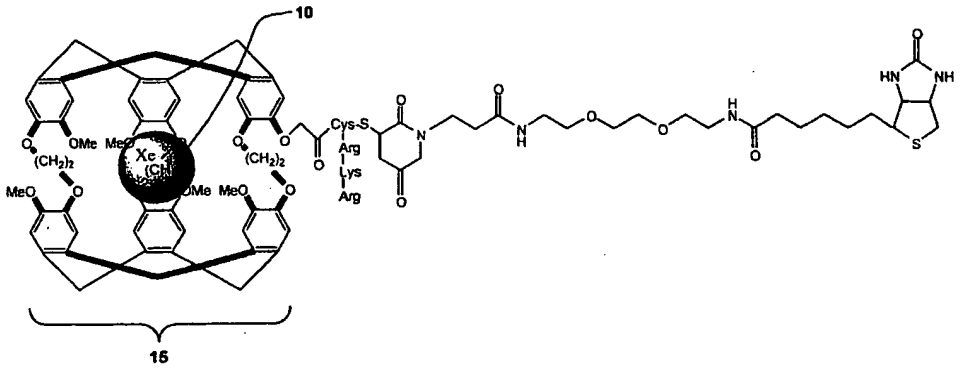
(54) Title: FUNCTIONALIZED ACTIVE-NUCLEUS COMPLEX SENSOR



(57) Abstract: A functionalized active-nucleus complex sensor that selectively associates with one or more target species. The functionalized active-nucleus complex comprises an active-nucleus and a targeting carrier. The targeting carrier comprises a first binding region having at least a minimal transient binding of the active-nucleus to form the funcitonalized active-nucleus complex that produces a detectable signal when the functionalized active-nucleus complex associates with the target species and a second binding region that selectively associates with the target species. Included is a method for assaying and screening for one or a plurality of target species utilizing one or a plurality of functionalized active-nucleus complexes with at least two of the functionalized active-nucleus complexes having an attraction affinity to different corresponding target species. The method comprises the steps of functionalizing an active-nucleus, for each functionalized active-nucleus complex, by incorporating the active-nucleus into a macromolucular or molecular complex that is capable of binding one of the target species. Then bringing the macromolecular or molecular complexes into contact with the target species and detecting the occurrence of or change in a nuclear magnetic resonance signal from each of the active-nuclei in each of the functionalized active-nucleus complexes in order to either monitor the occurrence of binding between each of the functionalized active-nucleus complexes and the target species or monitor a subsequent change in the environment of the target species after the binding occurs.

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FUNCTIONALIZED ACTIVE-NUCLEUS COMPLEX SENSOR

BACKGROUND OF THE INVENTION

1. Field of the Invention

A molecular or macromolecular structure and method of use are disclosed in which an active-nucleus is functionalized in at least a transient interaction with a target carrier to form a sensor that selectively associates with a target substrate or environment to produce a detectable signal. More specifically, a functionalized active-nucleus complex sensor is described in which an active-nucleus gas such as hyperpolarized xenon, hyperpolarized helium, or sulfur hexafluoride, or active-nuclei ^{19}F derivatives are bound in a carrier structure having a binding region specific for a target species. Upon binding to the target species the active-nucleus produces a detectable nuclear magnetic resonance signal or is detectable as a magnetic resonance imaging contrast agent. A plurality of target specific sensors may be utilized in the assaying and screening of samples containing the plurality of targets under either *in vivo* or *in vitro* conditions.

2. Description of the Background Art

The detection of biological molecules and their interactions is a significant component of modern biomedical research. In current biosensor technologies, simultaneous detection is limited to a small number of analytes by the spectral overlap of their signals. Recent biosensor technologies exploit surface plasmon resonance (1), fluorescence polarization (2), and fluorescence resonance energy transfer as detection methods (3). Although the sensitivity of such techniques is excellent, it has proven challenging to extend these assays to multiplexing capabilities because of the difficulty in distinguishing signals from different binding events. While nuclear magnetic resonance (NMR) spectroscopy is able to finely resolve signals from different molecules and environments, the spectral complexity and low sensitivity of NMR spectroscopy normally preclude its use as a detector of molecular targets in complex mixtures. Notable successes (4,5) in the application of NMR to such problems are still limited by long acquisition times or a limited number of detectable analytes. Laser polarized xenon NMR benefits from good signal to noise and spectral simplicity with the added advantage of substantial chemical shift sensitivity.

US Patent No. 5,642,625 discloses a high volume hyperpolarizer for spin-polarized noble gas. A method and apparatus are presented that allow spin exchange between atoms of the noble gas and an alkali metal such as rubidium.

Described in US Patent No. 5,785,953 is a magnetic resonance imaging technique using hyperpolarized noble gases as contrast agents. In particular, hyperpolarized xenon and helium are utilized in spatial distribution studies.

The foregoing references/patents reflect the state of the art of which the applicant is aware and are tendered with the view toward discharging applicant's acknowledged duty of candor in disclosing information which may be pertinent in the examination of this application. It is respectfully submitted, however, that none of these references/patents teach or render obvious, singly or when considered in combination, applicant's claimed invention.

SUMMARY OF THE INVENTION

An object of the present invention is to disclose a sensor and method of use comprising an active-nucleus (guest) and target carrier (host) that generates an NMR and/or MRI detectable signal upon association with a biological target.

Another object of the present invention is to relate a biosensor and method of *in vivo* and *in vitro* assaying/screening use that comprises a functionalize active-nucleus complex that selectively binds to and signals the presence of a desired biological target species.

A further object of the present invention is to describe biosensors and methods of *in vivo* and *in vitro* assaying/screening use that comprises a plurality of functionalize active-nucleus complexes with each complex selectively binding to and signaling the presence of a desired biological target species or analyte.

Still another object of the present invention is to present a biosensor and method of use in which the biosensor comprises an active-nucleus bound to a target carrier in which when the target carrier binds to a target species/analyte a detectable signal is produced upon the binding or upon alterations in the target species/analyte or its environment after the binding.

Yet a further object of the present invention is to disclose a plurality of biosensors and a multiplexed method of use in which each of the biosensors comprises an active-nucleus bound to a target carrier in which when the target carrier binds to a target species/analyte a detectable signal is produced upon the

binding or upon alterations in the target species/analyte or its environment after the binding, wherein all the biosensors' signals are simultaneously detectable.

Disclosed is a novel, functionalized active-nucleus sensor or biosensor that is directed to and signals the presence of a desired biological target species, often of biological origin or importance. An active-nucleus that presents a detectable signal to either nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) techniques is utilized in conjunction with a target specific carrier that interacts with both the active-nucleus and a biological target substrate or environment. The active-nucleus is capable of at least a minimal transient binding to a targeting carrier. The targeting carrier associates with the target substrate or environment, thereby stimulating the production of or change in the detectable signal from the active-nucleus in a functionalized interaction. "Functionalized" implies that when the active-nucleus is bound, in at least a minimal transient manner, by the targeting carrier, that the active-nucleus then responds to and signals the association between the targeting carrier and the target substrate or environment.

Since the basic subject invention enables the creation of several extremely powerful and versatile sensors and techniques that have eluded researchers for many years, a number of related embodiments are disclosed below. One requirement for the subject invention is that the reporter nucleus be sufficiently "active" or capable of producing a signal that is detectable by NMR or MRI techniques. Hyperpolarized noble gases such as xenon and helium meet this requirement, as do other nuclei such as ^{19}F, if present in sufficiently high concentrations. Thus, "active" implies that the nucleus generates a suitable signal that is capable of detection by NMR (either in strong or weak magnetic fields) and/or MRI contrast procedures. Several relatively standard techniques now exist for hyperpolarizing noble gases and include optical pumping or spin exchange procedures.

It is important to appreciate that for the subject invention the signal produced by the functionalized active-nucleus is studied directly to follow the behavior of the biological target substrate or environment. For example, xenon (as indicated above, other suitable active-nuclei are also contemplated as being within the realm of this disclosure), has a chemical shift that is enormously sensitive to its

local chemical environment. With the large xenon NMR signal created by optical pumping, the chemical shift can easily serve as a signature for the different chemical surroundings in which the xenon is found. Direct interaction between xenon and a target molecule has been observed by measuring the chemical shift and relaxation properties on xenon (in particular see, S. M. Rubin, M. M. Spence, B. M. Goodson, D. E. Wemmer, A. Pines, *Proceedings of the National Academy of Sciences of the United States of America* **97**, 9472-9475 (2000) that was part of the Provisional Application to which this application claims priority). However, the observation of this direct contact may be limited by the weak binding of xenon (or other suitable active-nuclei) to many target molecules of interest. To enhance the binding of the xenon, for example, to the biological target species/substrate/molecule/analyte of interest, and thus the population of xenon in contact with the target species/substrate/molecule/analyte, the xenon can be functionalized to strongly bind to the biological target species/substrate/molecule/analyte. This can be achieved by placing the xenon, or other suitable active-nuclei, in a target carrier that chemically recognizes and binds to the target. The target carrier has a first binding region that binds the xenon for at least a minimal transient period ("minimal" in the sense of a sufficiently long period of time to produce a useful signal) or, preferably, very strongly binds the xenon, and can not itself quickly relax the xenon polarization. Amplification of the sensing for both xenon and helium may be achieved by utilizing a "pool" of hyperpolarized active-nuclei atoms that either sense the environment by changes in the functionalized active-nucleus carrier complex (molecule, supramolecular, or microbubble environment) or else are in sufficiently rapid chemical exchange with active-nuclei in biosensor sites that are so sensitive, thereby amplifying the detection intensity yet further.

The target carrier has a second binding region that binds to or reacts with the target species/substrate/molecule/analyte. The target carrier allows xenon, or other active-nuclei, to be held in close proximity to the desired target, giving rise to a signal at a distinctive frequency indicating the presence of the target species/substrate/molecule/analyte. The functionalized active-nucleus/target carrier complex can "recognize" any one of a wide variety of biological target species/substrates/molecules/analytes (virtually an unlimited set of

organic/biomolecular structures) including biologically important species such as proteins, nucleic acids, carbohydrates, lipids, metabolites, and the like in either an *in vitro* or non-invasive *in vivo* setting at either high or low NMR utilized field strengths. For example, with diseases, the diagnostic power of the subject invention is quite clear. Various diseases present characteristic/defining targets such as unusual membrane proteins, lipids, or carbohydrates, unusual analytes in body fluids, and the like whose presence can easily be detected with the subject invention.

The subject method of assaying and screening for target species in *in vivo* and *in vitro* samples/subjects has many strengths, including the large signal to noise ratio afforded by the high polarization achieve with hyperpolarization of xenon, helium, and other suitable nuclei. With xenon, for example, there is a negligible natural presence of xenon, so there would be no interference from background xenon signals. In contrast to fluorescence (and other techniques that generate overlapping or interfering detection signals) assays and screening procedures, multiple functionalized active-nuclei tests are possible in one system (test-tube, plate, microplate, and the like), by creating active-nuclei carriers targeting different species/substrates/molecules/analytes or by altering the structure of the probe itself or both (see below). Each target would give rise to a separate active-nucleus chemical shift. These assays and screenings could also be carried out non-invasively *in vivo*, avoiding the exposure to radiation that radiometric assays and screenings require. In the case of optical pumping to create hyperpolarization of xenon and helium, because the large active-nuclei signals are generated by the optical pumping, high magnetic fields are unnecessary (as mentioned previously), and the chemical shifts can be detected in low magnetic fields using a superconducting quantum interference device (SQUID).

A preliminary calculation was performed (verified by the results obtained in Experimental Example #1, found below) to explore the initial feasibility of the subject technique *in vivo*. Based on capabilities of current spectroscopy, 200 nanomoles of nuclear spins are necessary to measure a signal. To compete with or match other forms of assays or screening procedures, 20 picomoles of target species must be detectable, A factor of 10^4 in signal is required. The

hyperpolarization compensates for at least a factor of 10^3, and the additional factor of ten is gained by the relatively simplicity of the spectrum, contrasted with a target (protein and the like) spectrum.

In its most basic configuration the subject invention comprises an active-nucleus and a target carrier that associates with both the active-nucleus and a desired target species to produce an detectable characteristic signal (typically a chemical shift or relaxation time for NMR or a contrast capability for MRI). The functionalized active-nucleus complex or subject biosensor that may comprise one or more identical or varied second binding regions. Additionally, the functionalized active-nucleus complex or subject biosensor may have varied first binding regions. Also, both the first and second binding regions could be varied within the same subject biosensor. As indicated, the subject invention allows a huge array of possible target species/substrates/molecules/analytes to be assayed/screened for in a parallel or multiplexing detection style within a single sample/subject.

Several possible active-nuclei gases exist, preferable hyperpolarized xenon and hyperpolarized helium, however, ^{19}F and similar nuclei, in sufficient concentration, are also contemplated. With fluorine atoms, an exemplary functionalized sensor comprises a target carrier having multiple fluorines such as a polyfluorinated dendrimer that selectively binds an organic target species/substrate/molecule/analyte or a form of fluorine such as sulfur hexafluoride trapped/bound within a functionalized (target specific binding) enclosing structure such as in "bubble" or "microbubble" environment as exemplified by a liposome, micelle, vesicle, bucky-ball type structures, natural and synthetic polymeric cages, and like. Conformational changes or alterations in the effective pressure on the "bubble" or "microbubble" would induce detectable signal variations from the subject biosensor. Variations in the immediate vicinity or environment of the biosensor should be detectable and include changes in ion concentrations, functioning of an ion channel, oxygen levels/distribution, neuron activity, and the like. It is noted that hyperpolarized xenon and hyperpolarized helium will also function as the signal reporting active-nuclei within similar functionalized "bubble" or "microbubble" structures.

The first binding region of the targeting carrier interacts/associates/binds with the active-nucleus. This first binding region includes structures such as

monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, carcerands, microbubbles, micelles, vesicles, molecular tennis balls, fullerenes, many general cage-like structures, and the like.

The second binding region in the targeting carrier comprises that portion of the subject biosensor that interacts with the target species/substrate/molecule/analyte. It is noted that multiple second binding regions are contemplated and may be identical or varied for attachment to a plurality of target sites.

The basic subject biosensor may contain additional useful components/structures. One or more "tether" regions may be included and serve to separate the first and second binding regions and to permit a region that may be further derivatized with additional moieties such as solubilizing regions. The solubilizing regions may contain polypeptides, carbohydrates, and other species that aid in solubilizing the subject probe.

More specifically, a functionalized active-nucleus biosensor is disclosed that capitalizes on the enhanced signal to noise, spectral simplicity, and chemical shift sensitivity of suitable active-nuclei gases (for example only and not by way of limitation, hyperpolarized xenon, hyperpolarized helium, and sulfur hexafluoride) and polyfluorinated containing species (utilized to target organic molecules) to detect specific targets. One subject sensor embodiment utilizes laser polarized xenon "functionalized" by a biotin-modified supramolecular cage, including a tether region having a solubilizing region, to detect biotin-avidin binding. This biosensor methodology can be used in analyte assays and screening procedures or extended to multiplexing assays for multiple analytes of screenings for multiple species.

Other objects, advantages, and novel features of the present invention will become apparent from the detailed description that follows, when considered in conjunction with the associated drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic model showing a first embodiment of the subject sensor illustrating a first binding region for holding the active-nucleus and a second binding region that associates with the target.

FIG. 2 is the schematic model sensor shown in FIG. 1 with a target species bound to the second binding region.

FIG. 3 is a schematic model showing a second embodiment of the subject sensor illustrating a first binding region identical to that depicted in FIG. 1 and a varied second binding region.

FIG. 4 is a schematic model showing a third embodiment of the subject sensor illustrating a varied first binding region and a second binding region identical to that depicted in FIG. 1.

FIG. 5 is a schematic model showing a fourth embodiment of the subject sensor illustrating both a varied first binding region and a varied second binding region, relative to those seen in FIG. 1.

FIG. 6 is a schematic model showing a fifth embodiment of the subject sensor illustrating a first binding region and a plurality of varied second binding regions.

FIG. 7 is a schematic model showing a sixth embodiment of the subject sensor illustrating a polyfluorinated first binding region and a second binding region that associates with an organic target species.

FIG. 8 is a schematic model showing a seventh embodiment of the subject sensor illustrating a first binding region containing sulfur hexafluoride and a second binding region.

FIG. 9A is a schematic model showing an eighth embodiment of the subject sensor illustrating a first binding region for holding the active-nucleus, a second binding region that associates with the target, and a tether region that connects the first and second binding regions.

FIG. 9B is the eighth sensor embodiment, seen in FIG. 9A, bound to a target species.

FIG. 10 is the eighth sensor embodiment, seen in FIG. 9A, illustrating an active-nuclei exchange process that enhances the generated detection signal.

FIG. 11 is a specific chemical structure of the subject sensor, without an active-nucleus, showing a first binding region (cryptophane-A) for holding the active-nucleus, a second binding region that associates with the target, a tether region that connects the first and second binding regions, and a solubilizing polypeptide attached to the tether.

FIG. 12 is the specific chemical structure shown in FIG. 11 with the active-nucleus xenon included within the cage-like, cryptophane-A, first binding region.

FIG. 13 shows ^{129}Xenon NMR spectra that monitors the binding of a biotin-functionalized xenon biosensor to avidin.

FIG. 14 shows the effect of cage structure (cryptophane-A in the top "A" view and cryptophane-E in the bottom "B" view) on the bound xenon chemical shift.

FIG. 15 is a schematic diagram showing multiplexing with functionalized xenon biosensors in which the top spectrum shows the three distinct functionalized xenon peaks, corresponding to different first binding region cages tethered to three second binding region ligands. The bottom spectrum shows the effect of adding the functionalized xenon biosensor to an unknown sample solution having targets.

DESCRIPTION OF THE PREFERRED EMBODIMENT

There are several preferred embodiments of the subject invention disclosed in the specification and depicted in FIGS. 1-15. In the subject invention's most basic configuration the subject invention comprises an active-nucleus (NMR or MRI detectable nuclei, preferably hyperpolarized xenon or hyperpolarized helium, however, ^{19}F is useful if present at sufficient levels) and a target carrier that associates with both the active-nucleus and a desired target to produce an detectable characteristic signal (typically a chemical shift or relaxation time for NMR or a contrast capability for MRI). FIG. 1 depicts the basic biosensor (the functionalized active-nucleus complex) 5 configuration and FIG. 2 shows the basic functionalized biosensor 5 bound to a target species/substrate/molecule/analyte 25. An active-nucleus 10 is bound in a targeting carrier. The targeting carrier comprises, at least, a first binding region 15, binding the active-nucleus, and a second binding region 20, wherein the second binding region 20 has a binding affinity for the target species/substrate/molecule/analyte 25 (the dashed line indicating the binding domain in the target). The detectable signal generated from the bound complex 5 in FIG. 2 is distinguishable from the detectable signal produced from the unbound complex 5 in FIG. 1 (see FIG. 13 for an equivalent signal shift).

As indicated above, one extremely useful characteristic of the subject invention is that the signal produced from the subject sensor is highly dependent upon its immediate environment and that signals created from similar, but not identical, sensors can be distinguished and utilized to detect multiple target species/substrates/molecules/analytes within the same sample. For example, FIG. 3 depicts a functionalized active-nucleus complex or subject biosensor 5' that has a varied second binding region 21, relative to the second binding region 20 seen in FIGS. 1 and 2. Thus, biosensor 5' would bind to a different target species/substrate/molecule/analyte or a different location on the original target species/substrate/molecule/analyte 25.

Additionally, FIG. 4 illustrates a functionalized active-nucleus complex or subject biosensor 5" that has a varied first binding region 16, relative to the first binding region 15 seen in FIGS. 1 and 2. Thus, biosensor 5" would generate a different signal than the signal produced by biosensor 5.

Also, both the first 16 and second binding regions 21 could be varied, relative to the biosensor seen in FIGS. 1 and 2, within the same subject biosensor to form biosensor 5"', as seen in FIG. 5. As indicated, the subject invention allows a huge array of possible target species/substrates/molecules/analytes to be assayed/screened for in a parallel or multiplexing detection style within a single sample.

FIG. 6 illustrates a subject biosensor that has several different second binding regions 20, 21, 22, and 23 attached to a first binding region producing sensor 5"". Sensor 5"" may bind to one or more targets via the presented second binding regions.

As indicated, several possible active-nuclei gases exist for any target species, preferable hyperpolarized xenon, hyperpolarized helium, and sulfur hexafluoride, however, ^{19}F, in sufficient concentration, is also contemplated for organic/biological targets. With fluorine atoms, an exemplary functionalized sensor comprises a target carrier having multiple fluorines such as a polyfluorinated dendrimer that selectively binds an organic/biological target species/substrate/molecule/analyte, as seen in FIG. 7. The polyfluorinated first binding region 35 may be a dendrimer or other suitable structure, including, but not limited to natural and synthetic polymers and the like. Additionally, sufficient

fluorine to produce an acceptable signal may be in the form of fluorine in sulfur hexafluoride and similar compounds. FIG. 8 illustrates sulfur hexafluoride 45 trapped/bound within a functionalized (target specific binding) enclosing structure 40 such as in "bubble" or "microbubble" environment as exemplified by a liposome, micelle, vesicle, bucky-ball type structures, natural and synthetic polymeric cages, and the like. A second binding region 20 is coupled to the enclosing structure 40 and binds the target. Conformational changes or alterations in the effective pressure on the "bubble" or "microbubble" would induce detectable signal variations from the active-nucleus in a subject biosensor. Variations in the immediate vicinity of the biosensor should be detectable and include such changes as: ion concentrations, oxygen levels, neuron activity, and the like. It is noted that hyperpolarized xenon and hyperpolarized helium will also function as signal reporting active-nuclei within similar functionalized "bubble" or "microbubble" structures.

It is noted that the subject targeting carrier comprises the first binding region (15 and 16 in FIGS. 1-6) that interacts/associates/binds with the active-nucleus. This first binding region includes structures such as monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, molecular tennis balls, fullerenes, many general cage-like structures, and the like. As long as structure or chemical nature of the first binding region permits effective signal producing interactions with the active-nucleus and binding to the target is not negated, a wide range of acceptable structures exists for this portion of the subject biosensor (the chemical shifts or relaxation times for the active-nucleus need to maintained as detectable).

Further, it is stressed that the second binding region (20, 21, 22, and 23 in FIGS. 1-6) in the targeting carrier comprises that portion of the subject biosensor that interacts with the target species/substrate/molecule/analyte. The first and second binding regions may be essentially identical, overlapping, or coextensive or separated by a plurality of atoms.

Clearly, the embodiments structures depicted in FIGS. 1-8 for the basic subject biosensor may contain additional useful components/structures. As seen in FIGS. 9A and 9B, one, or more. "tether" or "linker" or "spacer" regions 50 may

be included in the biosensor 6. Specifically, FIG. 9A shows a biosensor comprising a first binding region 15 for the active-nucleus, a bound active-nucleus 10, a second binding region 20 for the target, and a tether 50. The tether 50 serves to separate the first 15 and second 20 binding regions and may serve as a site where chemical modification can occur. FIG. 9B illustrates the binding of the second binding group 20 with a target 25. The chemical nature of the tether may be varied and includes polymethylenes, homo and heteropolymers, polyethers, amides, various functional group combinations, amino acids, carbohydrates, and the like. If desired, a plurality of tethered second binding groups may be bound to a first binding region, with each tether and/or second binding group the same or different.

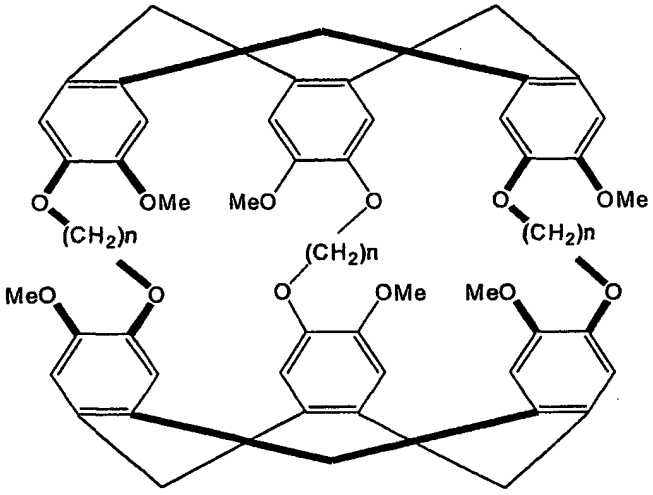
The tether may be derivatized to include a solubilizing region or other desired chemical feature such as additional binding sites and the like. The solubilizing region aids in solubilizing the biosensor in either a hydrophilic or hydrophobic environment. It is noted that a solubilizing region may also be included, either in addition to or separately, in the first and/or second binding regions. A water solubilizing region may include generally hydrophilic groups such as peptides, carbohydrates, alcohols, amines, and the like (for a specific example see FIGS. 11 and 12).

FIG. 10 illustrates a subject biosensor in which the signal is enhanced by a rapid chemical exchange of the active-nuclei. Free active-nuclei 11 rapidly exchange with the active-nucleus 10 bound in the first binding region 15 to produce an overall increase in sensitivity by enhancing the signal.

More specifically, a functionalized active-nucleus biosensor is disclosed that capitalizes on the enhanced signal to noise, spectral simplicity, and chemical shift sensitivity of a hyperpolarized xenon to detect specific biomolecules at the level of tens of nanomoles. Optical pumping (6) has enhanced the use of xenon as a sensitive probe of its molecular environment (7,8). Laser-polarized xenon has been utilized as a diagnostic agent for medical magnetic resonance imaging (MRI) (9) and spectroscopy (10), and as a probe for the investigation of surfaces and cavities in porous materials and biological systems. As indicated for an active-nucleus, xenon provides information both through direct observation of its NMR spectrum (11-17) and by the transfer of its enhanced polarization to surrounding

spins (18,19). In a protein solution, weak xenon-protein interactions render the chemical shift of xenon dependent on the accessible protein surface, and even allow the monitoring of the protein conformation (20). In order to utilize xenon as a specific sensor of target molecules the xenon was functionalized for the purpose of reporting specific interactions with the molecular target.

Specifically, a laser polarized xenon was "functionalized" by a biotin-modified supramolecular cage to detect biotin-avidin binding, thus, the specific target is avidin. Although, as previously indicated, the first binding region that holds the active-nucleus may be one of many possible structures, one suitable first binding region or cage is a member of the cryptophane family. Cryptophane has the following structure:

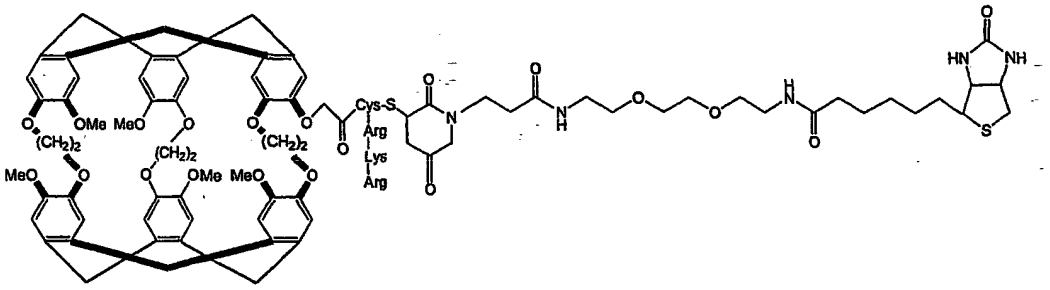


Formula I

Wherein n = 2 for cryptophane-A or n = 3 for cryptophane-E.

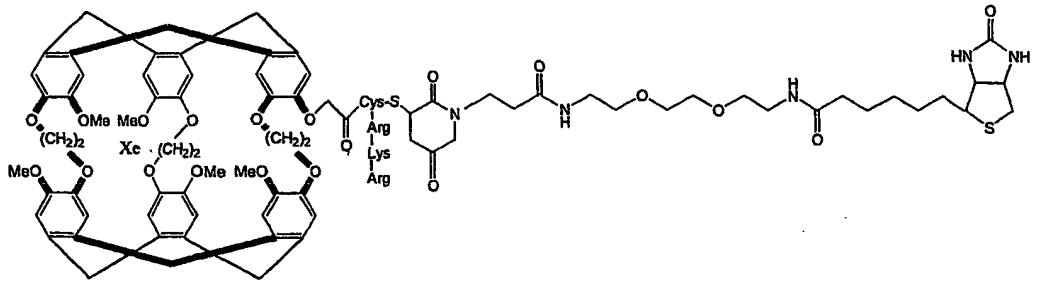
FIG. 11 (showing Formula II) depicts a specific targeting carrier in which the first binding region cryptophane-A 15 is covalently attached to a tether 50, having a solubilizing region 55, and biotin as the second binding region 20 (see the Example #1 below for synthesis details). The solubilizing region comprises a short

peptide chain (Cys-Arg-Lys-Arg) having positively charged groups at physiological pH values.



Formula II

FIG. 12 (showing Formula III) shows the functionalized active-nucleus biosensor when the xenon 10 is bound within the first binding region cryptophane-A 15 cage.



Formula III

EXPERIMENTAL EXAMPLE #1: FUNCTIONALIZED XENON AS A BIOSENSOR

By way of example and not by way of limitation, one embodiment of the subject invention comprises a functionalized system that exhibits molecular target recognition. FIGS. 11 (without xenon) and 12 (with xenon) show a biosensor molecule designed to bind both xenon and protein. Analogous to the general schematic diagrams seen in FIGS. 9A and 9B, the specifically synthesized subject biosensor molecule consists of three parts: the cage 15, which contains the xenon 10; the ligand 20, which directs the functionalized xenon 10 to a specific protein; and the tether 50, which links the ligand 20 and the cage 15. In this molecule, it is expected that the binding of the ligand 20 to the target protein (as in analogous FIG. 9B) will be reflected in a change of the xenon NMR spectrum.

The biotin (ligand second binding region 20) and avidin (target species) couple was chosen because of its high association constant (~10^{15} M^{-1}) (21) and the extensive literature characterizing binding properties of modified avidin or biotin (22). The cage 15 chosen for this embodiment was a cryptophane-A molecule (23) with a polar peptide chain (solubilizing region 55) attached in order to make the cryptophane-A water-soluble.

The cryptophane-A-based biosensor molecule was synthesized by a modified template directed procedure (23). Starting from 3,4-dihydroxybenxaldehyde and using allyl bromide to reversibly protect the meta-hydroxyl group (24), one of the 6 methoxyl groups in cryptophane-A was regioselectively replaced with a free hydroxyl group. Upon reacting with methyl bromoacetate followed by hydrolysis (25), the hydroxyl group in the modified cryptophane-A was converted to a carboxylic acid, which was subsequently coupled (using HOBt/HBTU/DIEA activation method) to the amino-terminus of a protected short peptide CysArgLysArg on rink amide resin. The resulting cryptophane-A-peptide conjugate was deprotected and cleaved off the resin using "Reagent K" (26), followed by purification with RP-HPLC (MicrosorbTM 80210C5, RP-C18 column, flow 4.5ml/min, buffer A: 0.1% TFA inH_2O, buffer B: 0.1% TFA in CH_3CN, linear gradient from 40% to 80% buffer B in 30 min). The purified conjugate was reacted with EZ-linkTMPEO-Maleimide activated biotin (Pierce) to

give the desired functionalized water-soluble cryptophane-A, which was further purified by RP-HPLC (same conditions). The last two peptide conjugated-products were verified by matrix-assisted laser desorption/ionization (MALDI)-time of flight-(TOF) mass spectrometry. All other intermediates were confirmed by ^{1}H NMR and MALDI-Fourier Transform mass spectrometry (FTMS).

Cryptophane-A has been shown to bind xenon with a binding constant $K \approx 10^{3}\ M^{-1}$ in organic solvents (15) but the affinity is likely to increase in aqueous solution because of the hydrophobic nature of xenon. The characteristic chemical shift for xenon inside a cryptophane-A molecule is very unusual for xenon dissolved in solution, approximately 130 ppm upfield from that of xenon in water. The only background xenon signal in the sample arises from xenon free in solvent, so the signal from the functionalized xenon is easily distinguishable. In the design of a xenon biosensor, a separate peak corresponding to xenon encapsulated by the cage is necessary, requiring both strong binding and a large difference between the xenon chemical shifts in the cage and solvent environments. The spin-lattice relaxation time for the functionalized xenon described herein was measured to be greater than 40 s, sufficient time for the required transfer, mixing, and detection of the polarized xenon.

The biosensor solution was prepared by dissolving ~0.5 mg of the cryptophane derivative (M.W.=2008 g mol^{-1}) in 700 μL of D_2O, yielding a concentration of ~300 μM. This concentration was consistent with absorbance measurements at 284 nm (ε_{284} = 36,000 $M^{-1}cm^{-1}$, determined for unmodified cryptophane-A by successive dilutions of a solution of known concentration). Approximately 80 nmol of affinity purified egg white avidin (Sigma) was used without further purification. Only half of the sample was located inside the detection region, so spectra actually reflect detection of ~40 nmol avidin monomer. Natural abundance xenon (Isotec) was polarized and introduced to the sample using previously described methods (16), showing ~5% polarization for the spectra shown in FIGS. 13 and 14. All NMR spectra displayed were obtained in single acquisition experiments at a nominal ^{129}Xe frequency of 82.981 MHz.

FIG. 13 shows the full ^{129}Xe NMR spectrum of the functionalized xenon in the absence of protein (the trace running near the bottom axis and having a far left peak and far right peaks). The far left peak at 193 ppm corresponds to xenon free in water while the far right peaks around 70 ppm are associated with xenon-bound cryptophane-A. The far right peaks are shown expanded in the center of FIG. 13, where the more intense, upfield peak (~70.7 ppm) corresponds to functionalized xenon and has a linewidth of 0.15 ppm (shown by the generalized schematic model, as seen in FIG. 9A). A smaller, middle peak (~71.5 ppm) approximately 1 ppm downfield of the functionalized xenon peak is attributed to xenon bound to a bare cage, without linker and ligand. As the unfunctionalized caged xenon does not interact specifically with the protein, it serves as a useful reference for the chemical shift and signal intensity of the functionalized xenon in the binding event.

Upon addition of ~80 nmol of avidin monomer, a third peak (~73 ppm) appears approximately 2.3 ppm downfield of the functionalized xenon peak, attributable to functionalized xenon bound to the protein. Correspondingly, the peak assigned to free functionalized xenon decreases in intensity relative to the reference peak while its position remains unchanged. The peak (~73 ppm) observed upon the addition of avidin is an unambiguous identifier of biotin-avidin binding, and hence the presence of avidin in solution.

The mechanism of the chemical shift change upon binding may result from actual contact between the cryptophane cage and the protein, leading to cage deformation and distortion of the xenon electron cloud. Changes in the rotational and vibrational motions of the cryptophane cage caused by binding to the protein could also affect the xenon chemical shift. Indeed, the sensitivity of xenon to perturbations of the first binding region cage is so great that deuteration of one methyl group results in a readily discernible change in the bound xenon chemical shift (17).

The subject methodology described herein offers the capability of multiplexing by attaching different second binding regions ligands to different first binding region cages, forming xenon sensors associated with distinct, resolved chemical shifts. As an example of this feature of the subject invention, FIG. 14

shows the changes in bound xenon chemical shift caused by using two different first binding region cages. The top spectrum A is that of xenon bound to cryptophane-A (n = 2 in Formula 1 above) in a tetrachloroethane solution and the lower spectrum B is that of xenon bound to cryptophane-E (n = 3 in Formula 1 above), similar to cryptophane-A, but with an additional methylene group added to each of the bridges between the caps. The resulting bound xenon chemical shift is ~30 ppm upfield from that of xenon bound to cryptophane-A. The linewidths for cryptophanes A and E are broadened by the exchange of xenon between the cage and tetrachloroethane, the organic solvent used.

The diagram in FIG 15 indicates schematically a multiplexing system (multiple functionalized xenon biosensors) for protein assay or screening procedures. The binding event assay/screening procedures would be distributed over a large chemical shift range by attaching each second binding region ligand to a different first binding region cage. In the absence of the targeted proteins, the spectrum, depicted in FIG. 15, would consist of three resolved xenon resonances because of the effect on the xenon chemical shift caused by cage modifications. Upon binding each of the targeted proteins, the xenon peaks should shift "independently," signaling each binding event and reporting the existence of and amount of protein present. As long as the differences in shift between xenon in the different cages exceed the shift change upon binding, it should be possible to monitor and assign multiple binding events. In FIG. 15, the top spectrum shows the three distinct functionalized xenon peaks, corresponding to different cages linked to three ligands. The bottom spectrum shows the effect of adding the functionalized xenon to an unknown solution. Upon addition to the unknown solution, the leftmost peak shifts entirely, representing the case in which all functionalized xenon is bound to its corresponding protein. The central peak decreases in intensity and a peak corresponding to the protein-bound functionalized xenon appears. The rightmost peak remains unaffected, indicating the absence of the corresponding protein target.

Thus, enabling experimental data for the subject functionalized active-nucleus biosensor has been disclosed that exploits the chemical shift of functionalized xenon upon binding to a target species/substrate/molecule/analyte.

The approach has several critical advantages over aspects of current biosensors, in that multiplexing assays and both heterogeneous and homogenous assays are possible. Furthermore, this methodology can be performed in biological materials *in vitro* or *in vivo* by combining the spatial encoding capabilities of MRI with the biosensing NMR capabilities of the functionalized xenon sensor. As indicated above, potential targets include, are not limited to, metabolites, proteins, toxins, nucleic acids, and protein plaques. It must be stated that, given the basic information presented herein, refinements of the subject functionalized detector molecules/sensors and the NMR procedures disclosed herein should further enhance the presented sensitivity by orders of magnitude, relative to the experimental example described herein and are within the realm of this disclosure.

The invention has now been explained with reference to specific embodiments. Other embodiments will be suggested to those of ordinary skill in the appropriate art upon review of the present specification.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

References

All of the following references are herein incorporated by reference. In particular, reference 16 (S. M. Rubin, M. M. Spence, B. M. Goodson, D. E. Wemmer, A. Pines, *Proceedings of the National Academy of Sciences of the United States of America* **97**, 9472-9475 (2000)) was in the original subject Provisional Application and is specifically incorporated herein by reference, as are all of the Provisional Application file documents.

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WHAT IS CLAIMED IS:

1. A macromolecule or molecular complex for use in assaying and screening for a biological target species or environment which:

a) contains a magnetically active nucleus;

b) is capable of binding the biological target species; and

c) gives rise to a magnetic resonance signal with a unique magnetic resonance property that:

i) occurs or changes with the occurrence of said binding event between the macromolecule or molecular complex and the biological target species and/or

ii) occurs or changes with a subsequent change in the environment of the biological target species after said binding occurs.

2. The macromolecule or molecular complex according to Claim 1, wherein said binding to the biological target species is either *in vivo* or *in vitro.*

3. The macromolecule or molecular complex according to Claim 1, wherein said macromolecule or molecular complex includes a structure selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and molecular cage structures.

4. The macromolecule or molecular complex according to Claim 1, wherein the macromolecule or molecular complex comprises a magnetically active gas contained within a molecular carrier.

5. The macromolecule or molecular complex according to Claim 4, wherein said magnetically active gas is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, and hyperpolarized helium.

6. The macromolecule or molecular complex according to Claim 1, wherein the macromolecule or molecular complex contains a self-assembled lipid complex.

7. The macromolecule or molecular complex according to Claim 6, wherein said self-assembled lipid complex is a liposome.

8. The macromolecule or molecular complex according to Claim 1, wherein the macromolecule or molecular complex is a rapidly exchanging complex between a macromolecule and a magnetically active gas.

9. The macromolecule or molecular complex according to Claim 8, wherein said magnetically active gas is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, and hyperpolarized helium.

10. The complex according to Claim 8, wherein said macromolecule is selected from a group consisting of cyclodextrins, cryptands, cryptophanes, carcerands, fullerenes, and molecular cage structures.

11. The macromolecule or molecular complex according to Claim 1, wherein said unique magnetic resonance property is selected from a group consisting of chemical shifts and relaxation times.

12. The macromolecule or molecular complex according to Claim 1, wherein said change in environment of the target species includes a change in pH, ion concentration, or concentration of other molecules near the target species.

13. A functionalized active-nucleus complex that selectively associates with a biological target species, wherein the functionalized active-nucleus complex comprises:

a) an active-nucleus and

b) a targeting carrier comprising:

i) a first binding region having at least a minimal transient binding of said active-nucleus to form the functionalized active-nucleus complex that produces a detectable signal when the functionalized active-nucleus complex associates with the target species and

ii) a second binding region that selectively associates with the target

species.

14. A functionalized active-nucleus complex according to Claim 13, wherein the functionalized active-nucleus complex is selected from a group consisting of a nuclear magnetic resonance reporter species and a magnetic resonance imaging contrast agent.

15. A functionalized active-nucleus complex according to Claim 13, wherein said active-nucleus is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, ^{19}F derivatives, and hyperpolarized helium.

16. A functionalized active-nucleus complex according to Claim 13, wherein said targeting carrier includes a structure selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and molecular cage structures.

17. A functionalized active-nucleus complex according to Claim 13, wherein said second binding region and said first binding region are coextensive or essentially the same structure.

18. A functionalized active-nucleus complex according to Claim 13, wherein:

a) said active-nucleus comprises hyperpolarized xenon and

b) said first binding region comprises a cryptophane.

19. A functionalized active-nucleus complex according to Claim 18, further comprising a solubilizing region associated with said targeting carrier.

20. A functionalized active-nucleus complex according to Claim 19, wherein said solubilizing region comprises a moiety that enhances the solubility of the functionalized active-nucleus complex in a desired environment.

21. A functionalized active-nucleus complex according to Claim 19, wherein said solubilizing region comprises at least one amino acid.

22. A functionalized active-nucleus complex according to Claim 18, further comprising a tether connecting said first and second binding regions.

23. A functionalized active-nucleus complex according to Claim 19, wherein said solubilizing region comprises a moiety bound to said tether.

24. A functionalized active-nucleus complex that selectively associates with a biomolecular target species, wherein the functionalized active-nucleus complex comprises:

a) an active-nucleus and

b) a targeting carrier comprising:

i) a first binding region having at least a minimal transient binding of said active-nucleus to form the functionalized active-nucleus complex that produces a detectable signal when the functionalized active-nucleus complex associates with the target species;

ii) a second binding region that selectively associates with the target species; and

iii) a tether region connecting said first and said second binding regions.

25. A functionalized active-nucleus complex according to Claim 24, wherein the functionalized active-nucleus complex is selected from a group consisting of a nuclear magnetic resonance reporter species and a magnetic resonance imaging contrast agent.

26. A functionalized active-nucleus complex according to Claim 24, wherein said active-nucleus is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, polyfluorinated derivatives, and hyperpolarized helium.

27. A functionalized active-nucleus complex according to Claim 24, wherein said targeting carrier includes a structure selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and molecular cage structures.

28. A functionalized active-nucleus complex according to Claim 24, wherein:

a) said active-nucleus comprises hyperpolarized xenon;

b) said first binding region comprises a cryptophane; and

c) said second binding region comprises biotin.

29. A functionalized active-nucleus complex according to Claim 24, further comprising a solubilizing region associated with said tether region.

30. A functionalized active-nucleus complex according to Claim 29, wherein said solubilizing region comprises a moiety that enhances the solubility of the functionalized active-nucleus complex in a desired environment.

31. A functionalized active-nucleus complex according to Claim 29, wherein said solubilizing region comprises at least one polar group.

32. A functionalized active-nucleus complex that selectively associates with at least one biological target species, wherein the functionalized active-nucleus complex comprises:

a) an active-nucleus and

b) a targeting carrier comprising:

i) a first binding region having at least a minimal transient binding of said active-nucleus to form the functionalized active-nucleus complex that produces a detectable signal when the functionalized active-nucleus complex associates with the target species;

ii) a plurality of second binding regions, wherein each of said second binding regions selectively associates with a target

species; and

iii) a plurality of tether regions wherein said first binding region is connected to each of said second binding regions by one of said plurality of said tether regions.

33. A method for assaying and screening for a biological target species which comprises:

a) functionalizing a magnetically active nucleus by incorporating said nucleus into a macromolucular or molecular complex that is capable of binding the target species;

b) bringing said macromolecular or molecular complex into contact with the target species; and

c) detecting the occurrence of or change in the nuclear magnetic resonance signal from said functionalized nucleus in order to:

i) monitor the occurrence of binding between said macromolecular or molecular complex and said target species and/or

ii) monitor a subsequent change in the environment of the target species after said binding occurs.

34. The method according to Claim 33, wherein said binding to said target species is either *in vivo* or *in vitro.*

35. The method according to Claim 33, wherein said macromolecule or molecular complex includes a structure selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and molecular cage structures.

36. The method according to Claim 33, wherein said macromolecular molecular complex includes a magnetically active gas contained within a molecular carrier.

37. The method according to Claim 36, wherein said magnetically active gas is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, and hyperpolarized helium.

38. The method according to Claim 33, wherein said magnetically active gas is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, hyperpolarized helium.

39. The method according to Claim 33, wherein said monitoring comprises detecting the occurrence of or change in a magnetic resonance signal with a unique magnetic resonance property.

40. The method according to Claim 39, wherein said magnetic resonance property is selected from a group consisting of chemical shifts and relaxation times.

41. The method according to Claim 33, wherein said change in environment of the biomolecular target comprises a change in pH, ion concentration, or concentration of other molecules near said target species.

42. A method for assaying and screening for a plurality of biological target species utilizing a plurality of functionalized active-nucleus complexes with at least two of the functionalized active-nucleus complexes having an attraction affinity to different corresponding biological target species, comprising the steps:

a) for each functionalized active-nucleus complex, functionalizing an active-nucleus by incorporating said active-nucleus into a macromolucular or molecular complex that is capable of binding one of said target species;

b) bringing said macromolecular or molecular complexes into contact with the target species; and

c) detecting the occurrence of or change in a nuclear magnetic resonance signal from each of said active-nuclei in each of said functionalized active-nucleus complexes in order to:

i) monitor the occurrence of binding between each of said functionalized active-nucleus complexes and said target species

and/or

ii) monitor a subsequent change in the environment of the target species after said binding occurs.

43. The method according to Claim 42, wherein said binding to said target species is either *in vivo* or *in vitro*.

44. The method according to Claim 42, wherein said functionalized active-nucleus complexes include structures selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and molecular cage structures.

45. The method according to Claim 42, wherein each said functionalized active-nucleus complex includes a magnetically active gas contained within a molecular carrier.

46. The method according to Claim 45, wherein said magnetically active gas is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, and hyperpolarized helium.

47. The method according to Claim 42, wherein said monitoring comprises detecting the occurrence of or change in a magnetic resonance signal with a unique magnetic resonance property from each said functionalized active-nucleus complex.

48. The method according to Claim 47, wherein said magnetic resonance property is selected from a group consisting of chemical shifts and relaxation times.

49. The method according to Claim 42, wherein said change in environment of the biomolecular target comprises a change in pH, ion concentration, or concentration of other molecules near said target species.

50. A method for assaying and screening for one or more biological target species which comprises:

a) functionalizing a magnetically active nucleus by incorporating said nucleus into a macromolucular or molecular complex that is capable of binding the target species;

b) bringing said macromolecular or molecular complex into contact with the target species; and

c) detecting the occurrence of or change in the nuclear magnetic resonance signal from said functionalized nucleus in order to:

i) monitor the occurrence of binding between said macromolecular or molecular complex and said target species and/or

ii) monitor a subsequent change in the environment of the target species after said binding occurs.

51. A biosensor, comprising:

a) an environment targeting agent having an attraction affinity to a chemical environment; and

b) an active-nucleus carried by said environment targeting agent, wherein said environment targeting agent is capable of recognizing a change in said chemical environment and a detectable signal from said active-nucleus indicates said change in said chemical environment.

52. A biosensor according to Claim 51, wherein said environment targeting agent comprises an active-nucleus binding region for carrying said active-nucleus and an environment recognition region, wherein said active-nucleus binding region is selected from a group consisting essentially of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, carcerands, microbubbles, micelles, vesicles, fullerenes, and general molecular cage structures.

53. A biosensor according to Claim 51, wherein said active-nucleus is selected from a group consisting essentially of hyperpolarized xenon, sulfur hexafluoride, and hyperpolarized helium.

54. A biosensor according to Claim 51, wherein recognition of said chemical environment by said environment targeting agent produces a detectable chemical shift from said active-nucleus.

55. A biosensor according to Claim 51, wherein recognition of said chemical environment by said environment targeting agent produces a magnetic resonance signal.

56. A biosensor according to Claim 51, wherein said change in said chemical environment is selected from a group consisting of ion channel functioning, neuron functioning, ion binding and transport, and oxygen distribution.

57. A biosensor mixture, comprising a plurality of functionalized active-nucleus complexes, at least two of the functionalized active-nucleus complexes having an attraction affinity to different corresponding target species, wherein each of said functionalized active-nucleus complexes comprises:

a) an active-nucleus and

b) a targeting carrier comprising:

i) a first binding region having at least a minimal transient binding of said active-nucleus to form the functionalized active-nucleus complex that produces a detectable signal when the functionalized active-nucleus complex associates with the target species and

ii) a second binding region that selectively associates with the target species.

58. A biosensor mixture according to Claim 57, wherein each of the functionalized active-nucleus complexes is selected from a group consisting of a nuclear magnetic resonance reporter species and a magnetic resonance imaging contrast agent.

59. A biosensor mixture according to Claim 57, wherein each of said active-nuclei is selected from a group consisting of hyperpolarized xenon, ^{19}F derivatives, sulfur hexafluoride, and hyperpolarized helium.

60. A biosensor mixture according to Claim 57, wherein each of said targeting carriers includes a structure selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and molecular cage structures.

61. A biosensor mixture according to Claim 57, wherein each of said second binding regions and said first binding regions are coextensive or essentially the same structure.

62. A biosensor mixture according to Claim 57, wherein:

a) said active-nucleus comprises hyperpolarized xenon and

b) said first binding region comprises a cryptophane.

63. A biosensor mixture according to Claim 57, wherein each said targeting carrier further comprises a solubilizing region associated with each said targeting carrier.

64. A biosensor mixture according to Claim 63, wherein each said solubilizing region comprises a moiety that enhances the solubility of the functionalized active-nucleus complex in a desired environment.

65. A biosensor mixture to Claim 64, wherein each said solubilizing region comprises at least one amino acid.

66. A biosensor mixture according to Claim 57, wherein each said functionalized active-nucleus complex further comprises a tether connecting said first and second binding regions.

67. A biosensor mixture according to Claim 66, wherein each said functionalized active-nucleus complex includes a solubilizing region bound to said tether.

68. A biosensor mixture, comprising:

a) a plurality of functionalized active-nucleus complexes, at least two of said functionalized active-nucleus complexes having an attraction affinity to different corresponding chemical environments and

b) an active-nucleus carried by each of said functionalized active-nucleus complexes, wherein each said active-nucleus produces a detectable signal in said chemical environment.

69. A biosensor mixture according to Claim 68, wherein each said functionalized active-nucleus complexes includes a targeting carrier that is selected from a group consisting of monoclonal antibodies, dendrimers, self-assembled lipid complexes, liposomes, cyclodextrins, cryptands, cryptophanes, carcerands, microbubbles, micelles, vesicles, fullerenes, and general molecular cage structures.

70. A biosensor mixture according to Claim 68, wherein each said active-nucleus is selected from a group consisting of hyperpolarized xenon, sulfur hexafluoride, and hyperpolarized helium.

71. A biosensor mixture according to Claim 68, wherein said detectable signal is an NMR chemical shift.

72. A biosensor mixture according to Claim 68, wherein said detectable signal is a magnetic resonance signal.

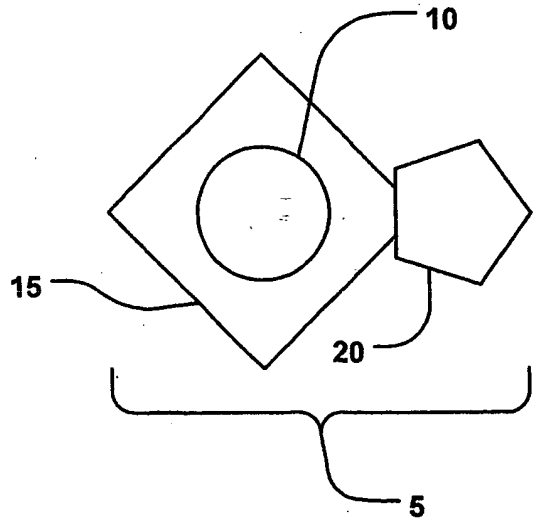

FIGURE 1

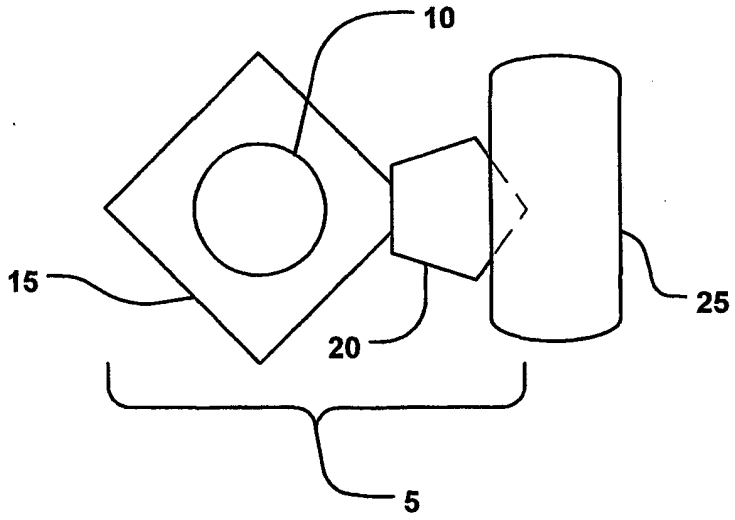

FIGURE 2

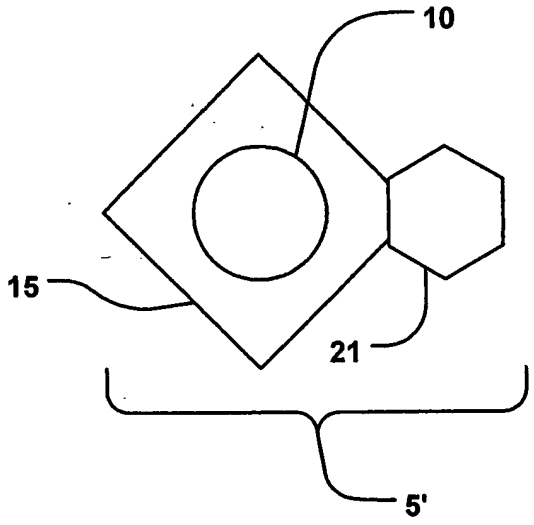

FIGURE 3

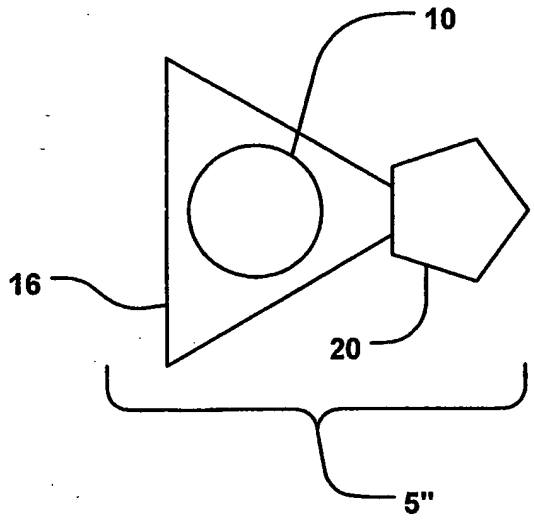

FIGURE 4

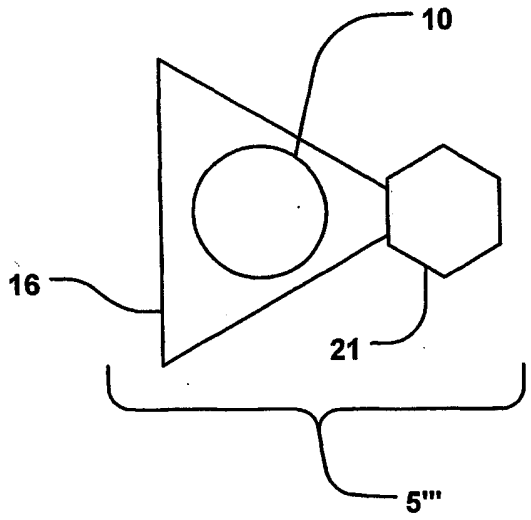

FIGURE 5

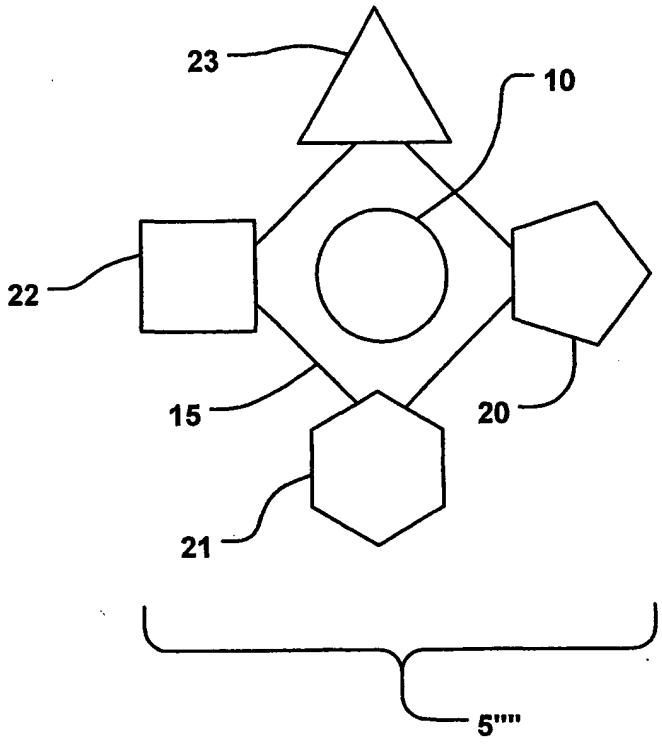

FIGURE 6

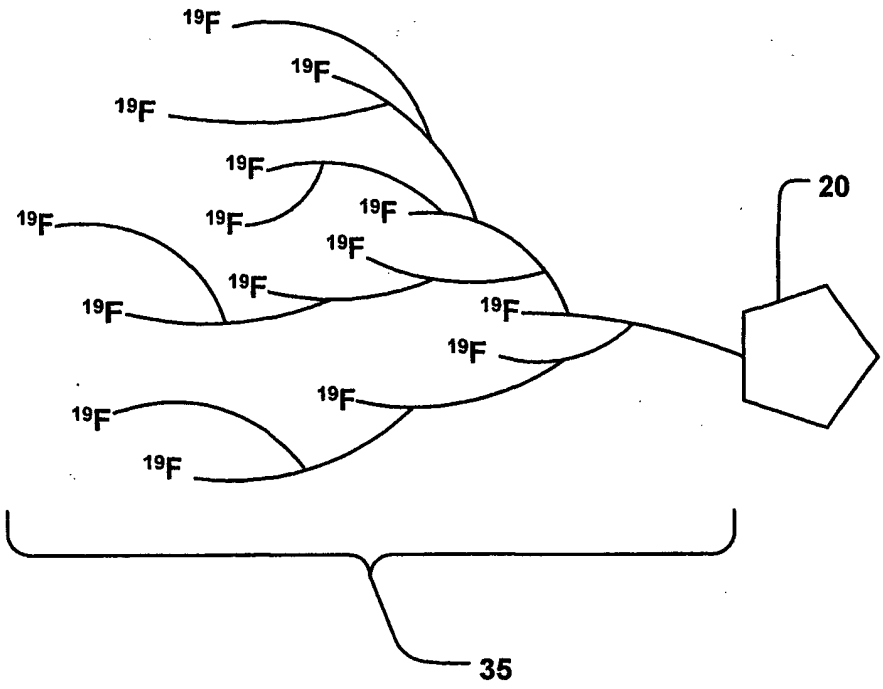

FIGURE 7

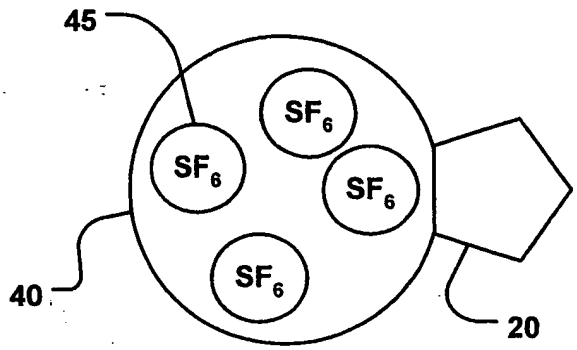

FIGURE 8

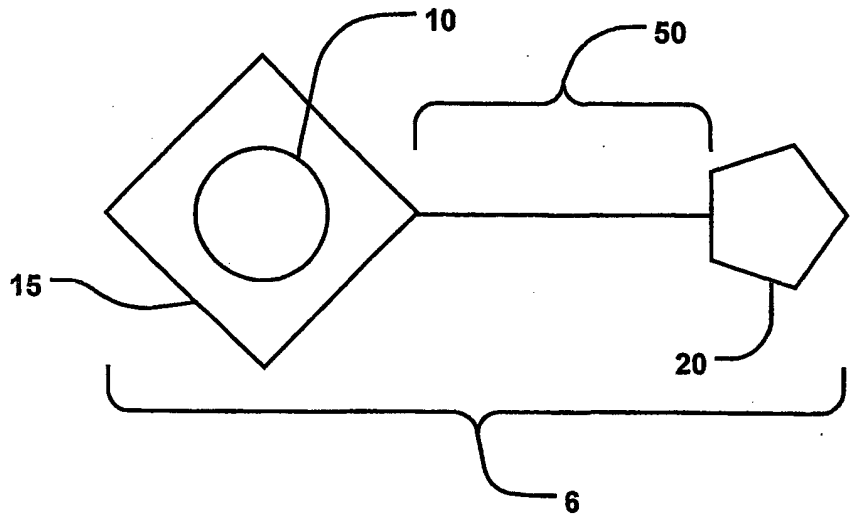

FIGURE 9A

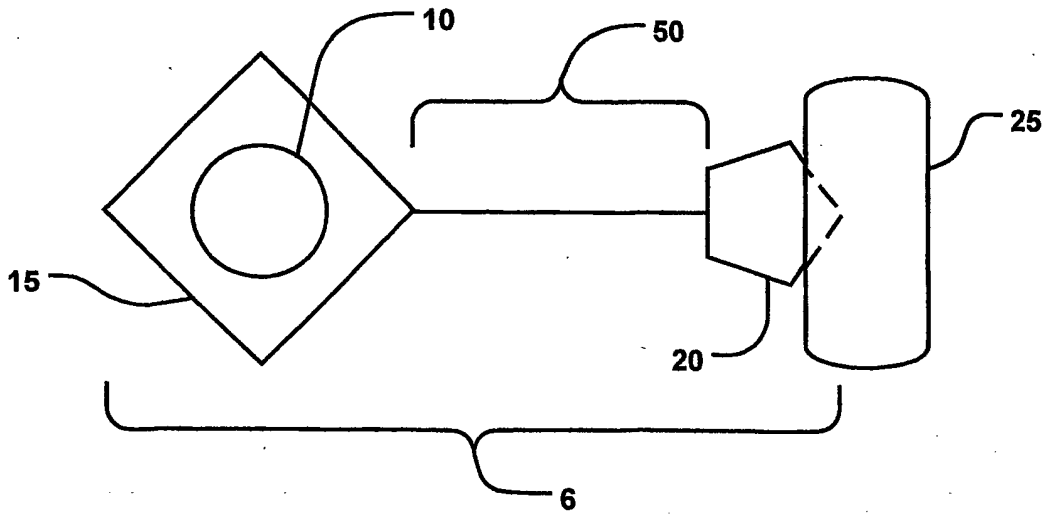

FIGURE 9B

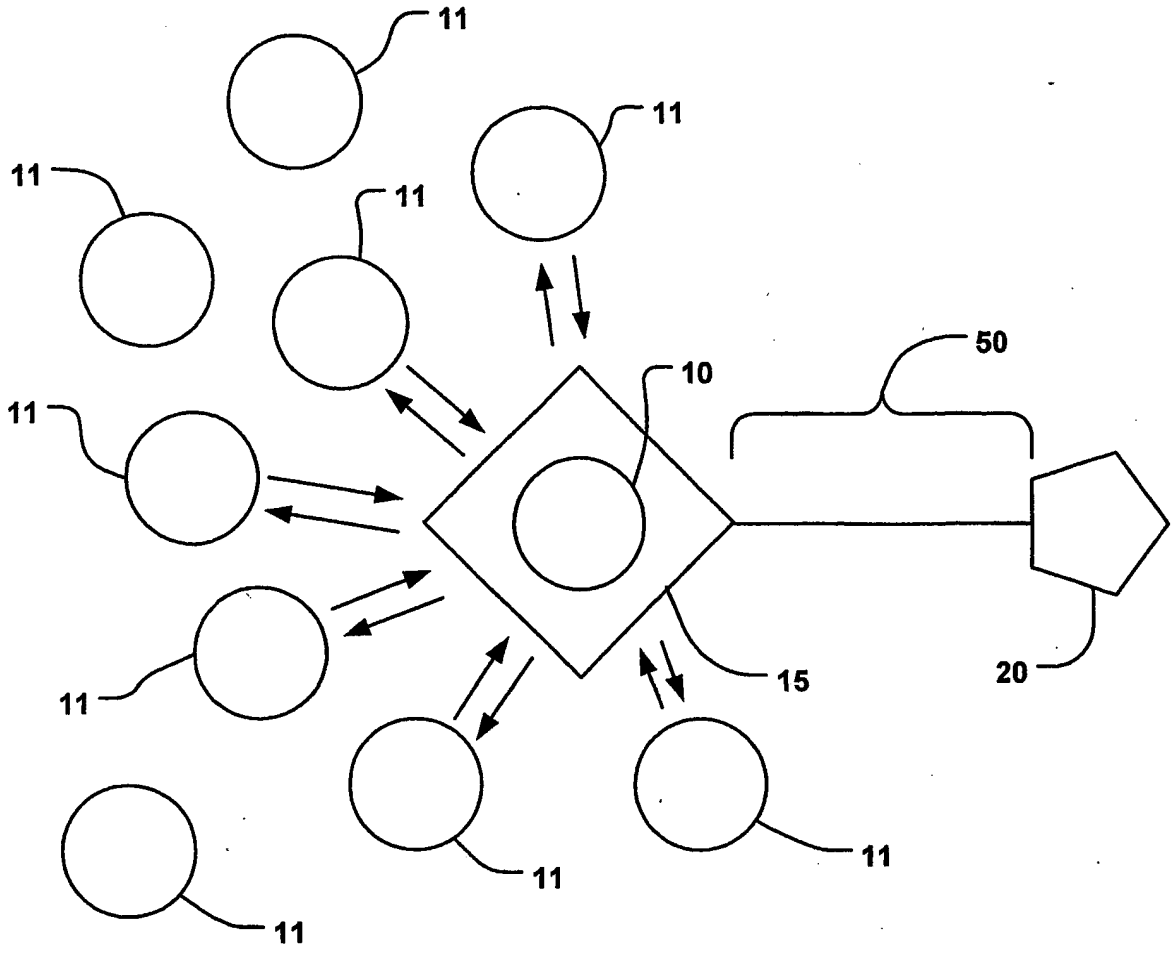

FIGURE 10

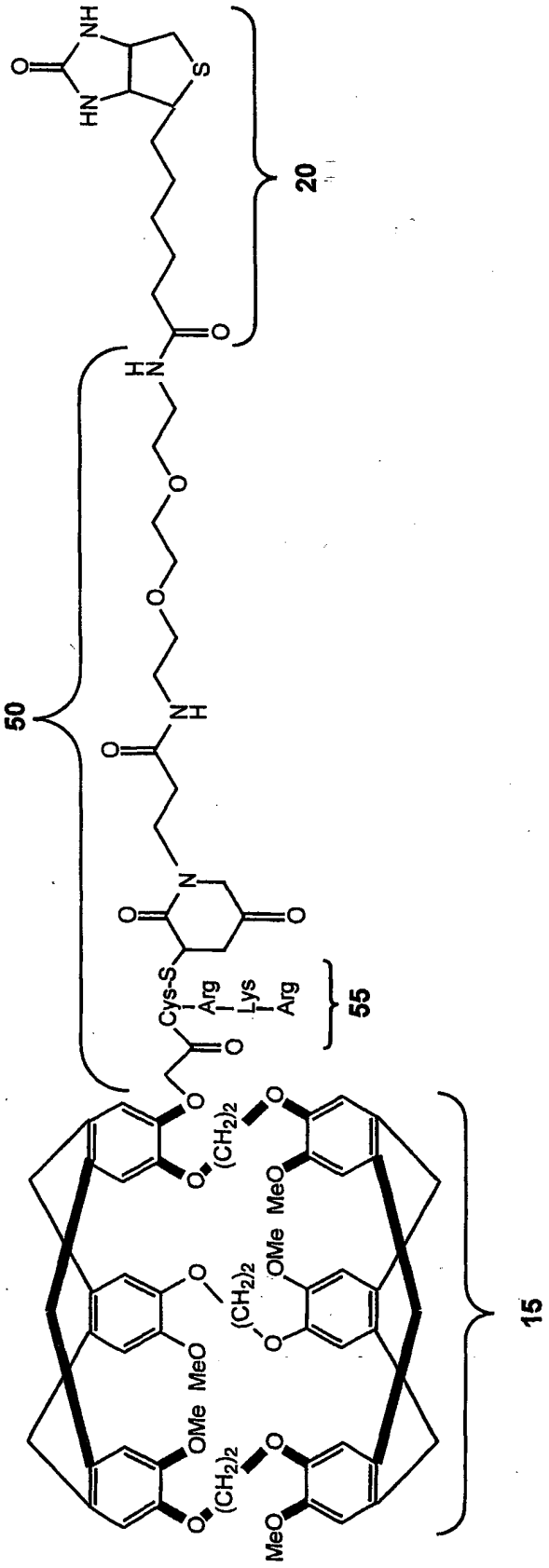

FIGURE 11

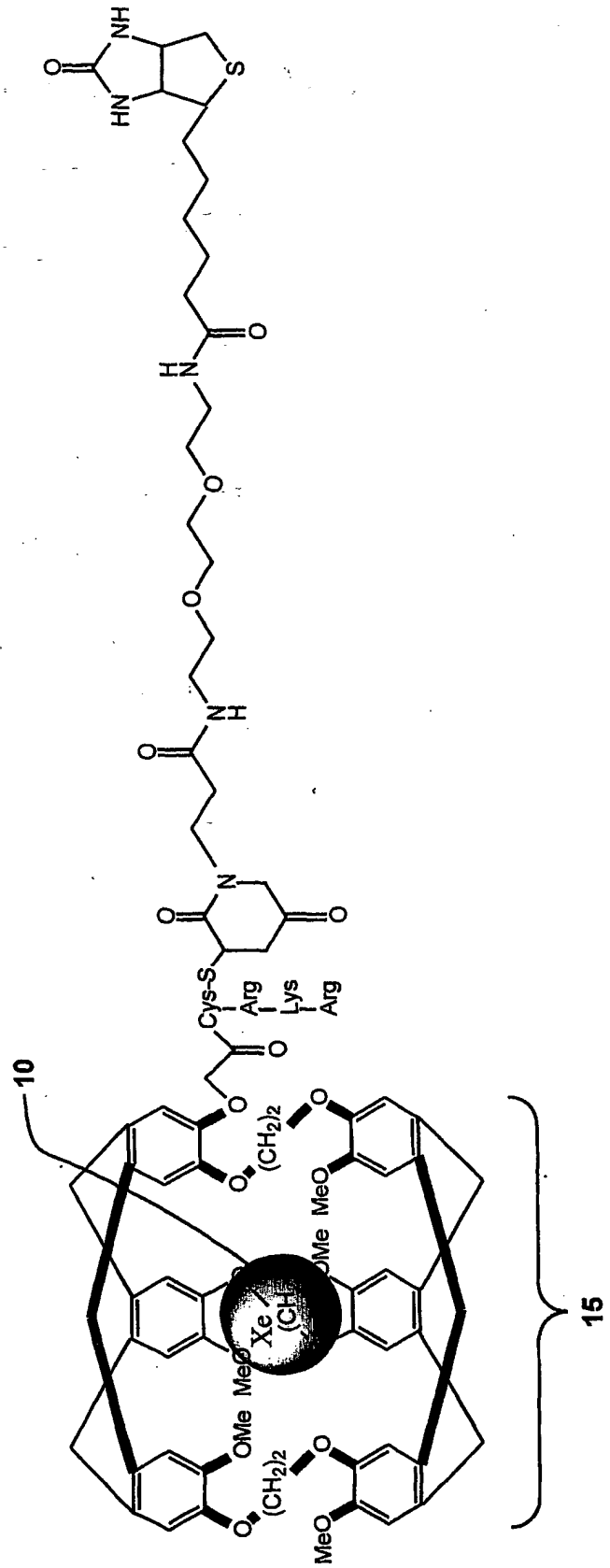

FIGURE 12

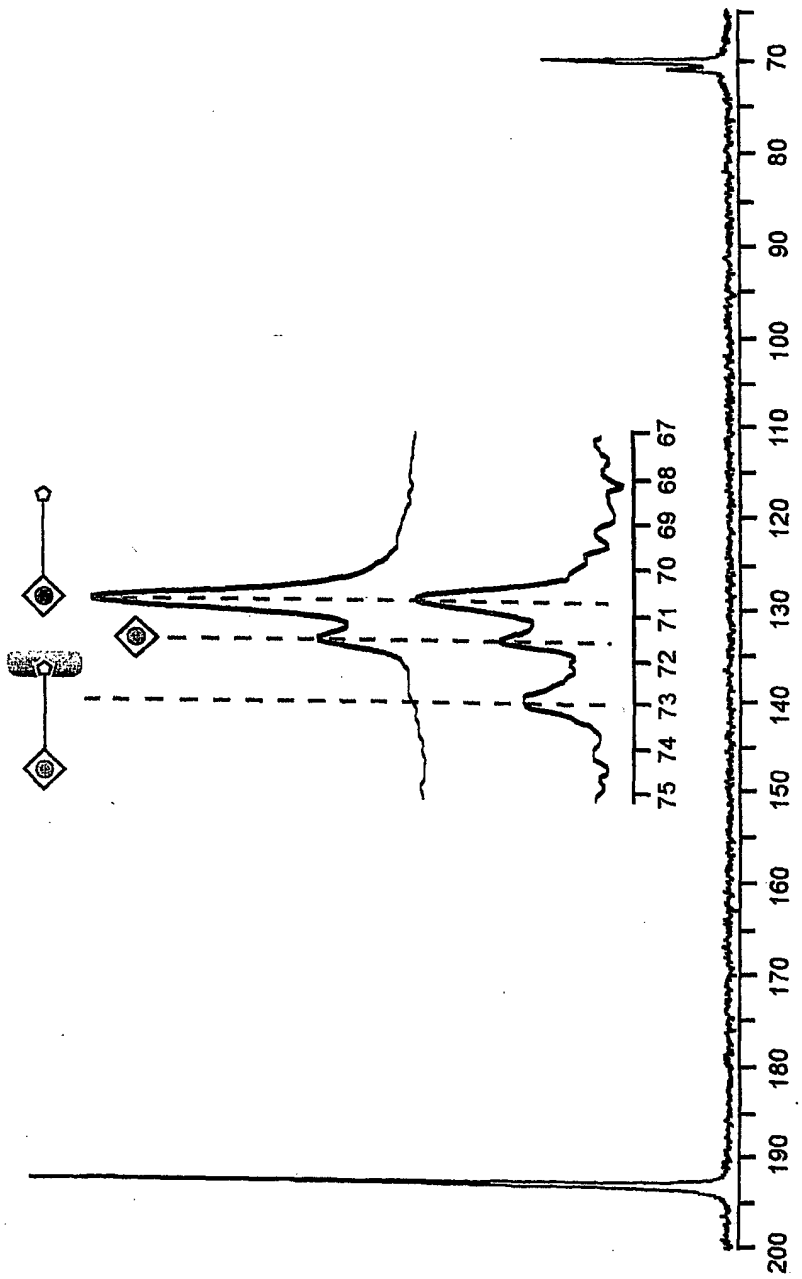
70 80 90 100 110 120 130 140 150 160 170 180 190 200
chemical shift (ppm)
67 68 69 70 71 72 73 74 75

FIGURE 13

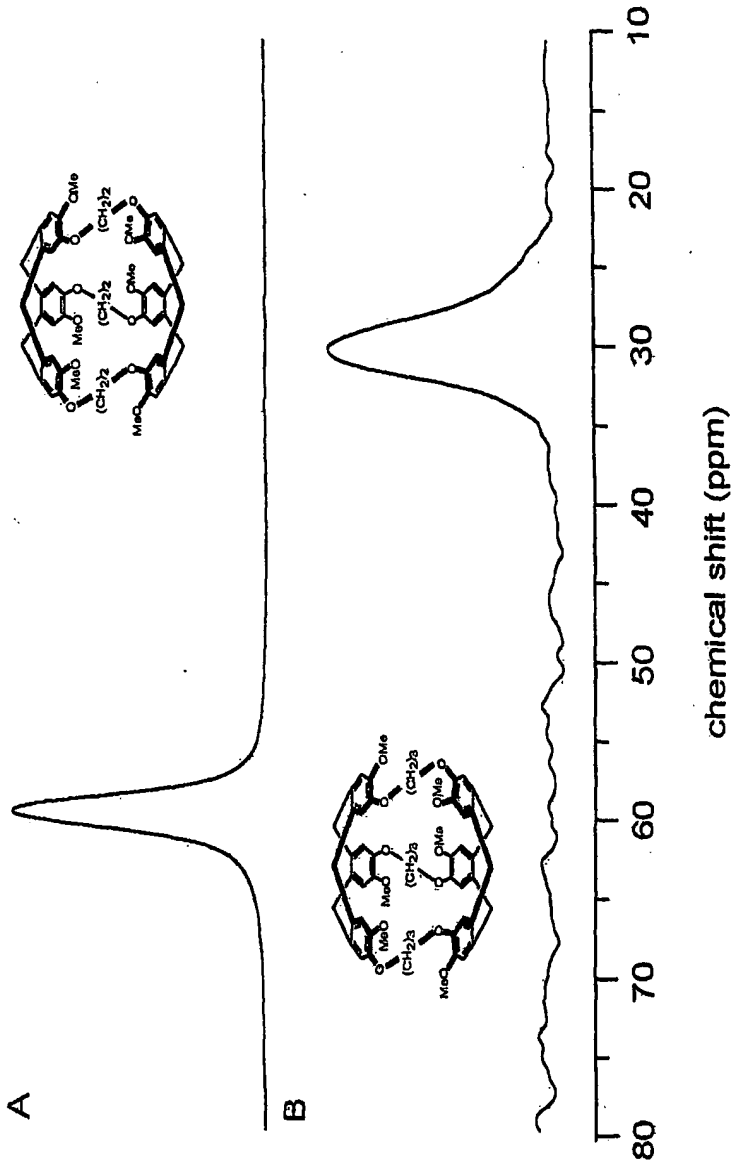

FIGURE 14

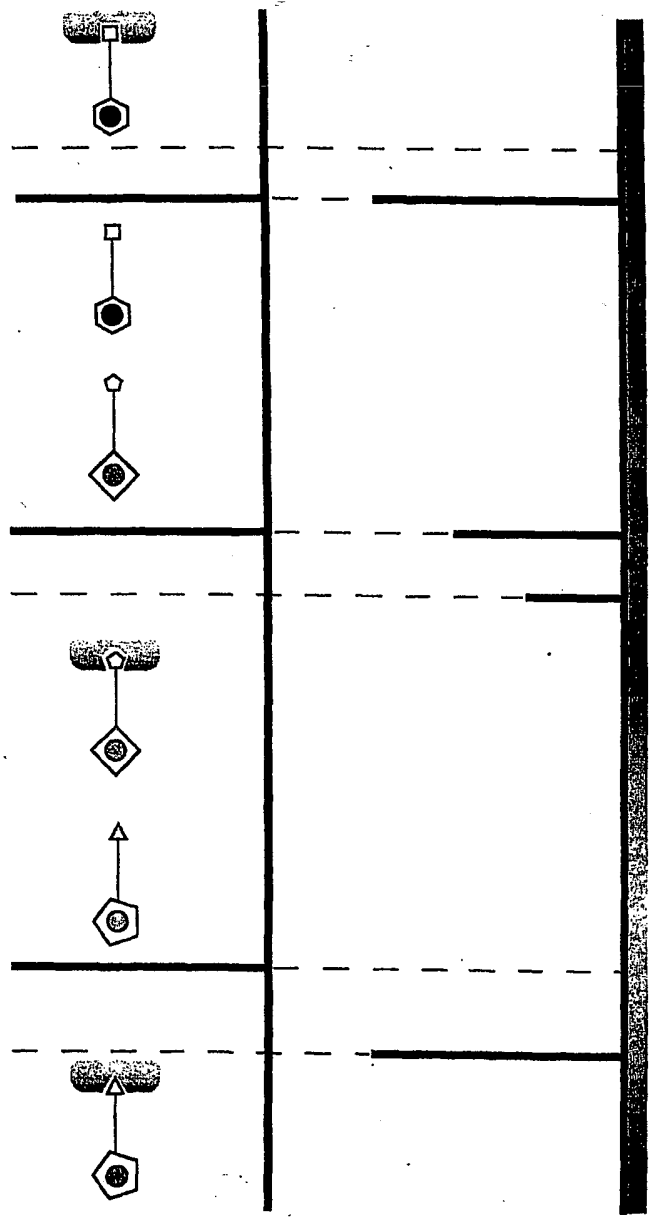

FIGURE 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/22050

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/045, 31/05, 49/06, 49/12, 49/18; A61B 5/055

US CL :424/9.321, 9.3222, 9.34; 514/728, 736, 738

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.321, 9.3222, 9.34; 514/728, 736, 738

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, CAPlus, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	BROTIN et al. 129 Xe NMR Spectroscopy of Deuterium-Labeled Cryptophane-A Xenon Complexes: Investigation of Host-Guest Complexation Dynamics. J. Am. Chem. Soc. January 2000, Vol. 122, pages 1171-1174, especially page 1174	1-6, 8-12 --------- 13-72
Y	BARTIK et al. 129 Xe and 1 H NMR Study of the REversible Trapping of Xenon by Cryptophane-A in Organic Solution. J. Am. Chem. Soc. January 1998, Vol. 120, pages 784-791, especially 784-785.	1-72
X Y	US 6,051,208 A (JOHNSON et al) 18 April 2000, col. 3-6.	33-50 ----- 1-32, 51-72

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 SEPTEMBER 2001	Date of mailing of the international search report 31 DEC 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence For SHAHNAM SHARAREH Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/22050

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	US 5,785,953 A (ALBERT et al) 28 July 1998, abstract, col 19-26.	33-55, 68-72 ---------- 1-32, 56-67
Y	US 6,042,809 A (TOURNIER et al) 28 March 2000, abstract, col 9-12.	1-72
Y	US 5,846,517 A (UNGER et al) 08 December 1998, col. 32-39, col. 58-62.	1-72
A	FARUQI et al. Structure-Function Analysis of Protease-activated Receptor 4 Tethered Ligand Pepeptides. The Journal Of Biological Chemistry. 30 June 2000, Vol. 275, No. 26, pages 19728-19734.	1-72
A	US 6,023,162 A (JOHNSON) 08 February 2000, abstract, col 2-4.	1-72
A	US 5,642,625 A (CATES, JR et al) 01 July 1997, col 30-34.	1-72

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/22050

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/22050

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-12, 33-41, drawn to macromolecules or molecular complex and methods of use thereof.
Group II, claim(s) 13-32, drawn to functionalized active-nucleus complex having a tether region connecting a first and a second binding region of a targeting carrier.
Group III, claim(s) 51-56, drawn to a biosensor.
Group IV, claims 57-72, 42-50, drawn to a biosensor mixture comprising at least two of a functionalized active-nucleus complexes comprising a targeting carrier having two binding regions, and methods of use thereof.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-IV are independent from one another because they claims and utilize compositions which have different components (i.e. different targeting carrier components) and thus are of different scope.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

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24 January 2002 (24.01.2002)

PCT

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WO 02/05803 A1

(51) International Patent Classification[7]: A61K 31/045, 31/05, 49/06, 49/12, 49/18, A61B 5/055

(21) International Application Number: PCT/US01/22050

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/218,549 13 July 2000 (13.07.2000) US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th Floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

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(74) Agent: O'BANION, John, P.; O'Banion & Ritchey LLP, Suite 1550, 400 Capitol Mall, Sacramento, CA 95814 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

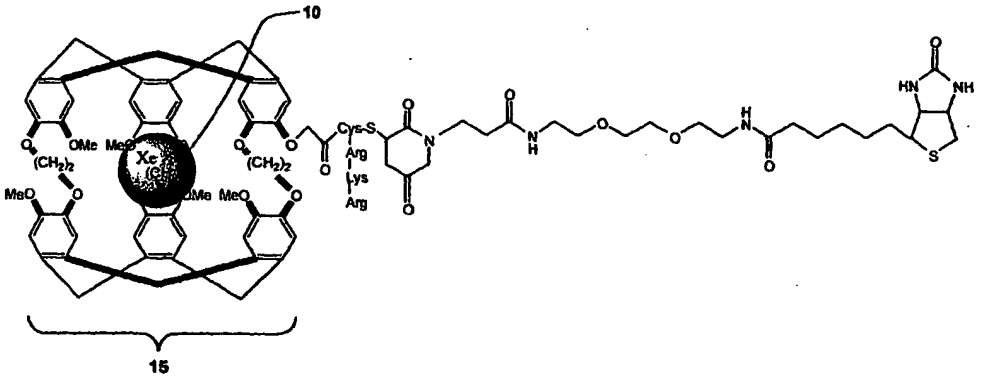
Published:
— *with international search report*

(48) Date of publication of this corrected version:
21 March 2002

(15) Information about Correction:
see PCT Gazette No. 12/2002 of 21 March 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FUNCTIONALIZED ACTIVE-NUCLEUS COMPLEX SENSOR



(57) Abstract: A functionalized active-nucleus complex sensor that selectively associates with one or more target species. The functionalized active-nucleus complex comprises an active-nucleus and a targeting carrier wherein the targeting carrier comprises a first binding region having at least a minimal transient binding of the active-nucleus to form the functionalized active-nucleus complex that produces a detectable signal when the functionalized active-nucleus complex associates with the target species and a second binding region that selectively associates with the target species. Included is also method for assaying and screening for one or a plurality of target species utilizing one or a plurality of functionalized active-nucleus complexes with at least two of the functionalized active-nucleus complexes having an attraction affinity to different corresponding target species.

WO 02/05803 A1